

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800

Open access books available

122,000

International authors and editors

135M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



---

# Physiology and Pathology of Mitochondrial Dehydrogenases

---

Andrea M.P. Romani

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.76403>

---

## Abstract

Effective mitochondria bioenergetics requires the proper functioning of various intra-organellar dehydrogenases. By providing pyridine and flavin adenine dinucleotides to the electron chain, or Acyl-CoA for the reactions of the tris-carboxylic acid cycle, or the acylation of fatty acids to undergo  $\beta$ -oxidation, these dehydrogenases preside to the organelle production of ATP, required for a variety of cellular functions under physiological conditions. The operation of the various dehydrogenases is mainly regulated by hormones through changes in intra-mitochondrial cation levels and ratios, namely  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ . Dysregulation of specific dehydrogenases under pathological conditions results in marked changes in the energetic level of the organelle and ultimately the cell. The present review will attempt to highlight the role of the main mitochondrial dehydrogenases and their regulation, and provide a general assessment of their dysfunction and associated consequences under some of the most common human pathologies.

**Keywords:** mitochondria, dehydrogenases, calcium, magnesium, diabetes, obesity, cancer

---

## 1. Introduction

The oldest reports of intracellular structures that most likely represented mitochondria date back to 1840s [1], whereas the association of these organelles with specific biological functions essential for the hosting cells can be attributed to Altman [2]. The first utilization of the term 'mitochondrion' is attributed to Benda, in 1895 [3]. Regaud, in the early twentieth century, suggested that the organelle was constituted of protein and lipids [4], but it is not until the 1960s through the seminal work by Palade [5] and the use of electron microscopy that the structure and morphology of the organelle as it is currently known was defined. Alongside

these studies, the utilization of various experimental approaches resulted in the identification of a variety of enzymes and proteins localized either within the mitochondrial matrix or associated with the inner mitochondrial membrane or the inter-membraneous space, as well as the presence of mitochondrial DNA [for an historical overview see [6] and the refs. therein]. Because the reduplication of mitochondrial DNA occurs following the reduplication of cellular DNA, and mtDNA only accounts for less than half of all the mitochondrial enzymes and proteins, sophisticated biological mechanisms are in place to coordinate the import and proper allocation of mitochondrial enzymatic components within the organelle following their cellular synthesis. Furthermore, mitochondria are not static organelles but undergo constant fusion and division (fission) to form ever changing tubular networks in most eukaryotic cells. These changes are important for the normal physiology of the cell, and may dramatically affect cell behavior under pathological conditions.

From the functional stand-point, mitochondria are regarded as the source of energy for the cell, in that they produce a steady flow of ATP that is utilized in a variety of cellular functions and signaling events. In addition to producing ATP, mitochondria play a major role in signaling, cell differentiation and growth, cell cycle, cell death (apoptosis), and in the production of reactive oxygen species (ROS) [7].

While certain mitochondrial functions are present almost exclusively in specific cells (e.g. ammonia detoxification in hepatic mitochondria), ATP production represents the predominant and ubiquitous function of the organelle in all eukaryotic cells. The chemiosmotic coupling of proton movement out of specific sites of the electron transport chain to their re-entry through the F<sub>0</sub>-F<sub>1</sub> ATPase, with associated ATP production in a 3H<sup>+</sup>: 1ATP ratio, as proposed by Mitchell [8] in 1966, is now generally accepted, although some details of the process need further refinement. The generated ATP is then extruded in a 1ADP<sub>in</sub>: 1ATP<sub>out</sub> ratio into the cytoplasm through the adenine nucleotide translocase (AdNT), one of the most abundant proteins present in the mitochondrial membrane.

Despite our current understanding of the mechanisms responsible for ATP synthesis within the mitochondria and its extrusion across the organelle membrane, the specific role of various mitochondrial dehydrogenases and their regulation in modulating ATP synthesis to maintain a stable and viable flow of energetic 'currency' for the cell based on the available substrate is still not completely elucidated.

The present review does not have the pretense of being comprehensive in addressing the role and regulation of all mitochondrial dehydrogenases. Rather, it will focus on the regulation of some of them, and the dysregulation occurring under specific pathological conditions, which ultimately impacts the proper functioning of specific organs or tissues.

## 2. Key mitochondrial dehydrogenases and their regulation

For the purpose of this review, we will focus on the following dehydrogenases:

*Glutamate dehydrogenase* (EC 1.4.1.4): The presence of this enzyme in eukaryotes is essential for urea synthesis in the urea cycle, in that it converts glutamate to  $\alpha$ -ketoglutarate, and

vice versa. However, in addition to having a very low  $K_m$  for ammonia ( $\sim 1$  mM), at equilibrium the reaction catalyzed by this enzyme favors ammonia and  $\alpha$ -ketoglutarate production. Conversion of  $\alpha$ -ketoglutarate to glutamate does occur in brain mitochondria as a result of local  $NAD^+ : NADH/H^+$  ratio [9]. In humans, the activity of the enzyme is regulated through ADP-ribosylation, but also by caloric restriction or hypoglycemia, as either of the latter two conditions increase glutamate dehydrogenase activity to increase the amount of  $\alpha$ -ketoglutarate produced. In turn,  $\alpha$ -ketoglutarate is used to provide energy through the citric acid cycle, ultimately generating ATP. The activity of the enzyme does not appear to be regulated by an increase in the levels of  $Ca^{2+}$  and  $Mg^{2+}$ , which can accumulate to a significant extent within mitochondria under physiological conditions. On the other hand,  $Zn^{2+}$  has been reported to act as an allosteric regulator of the enzyme, together with ATP and possibly GTP.

*$\alpha$ -ketoglutarate dehydrogenase (1.2.4.2)*: Also known as oxoglutarate dehydrogenase complex (OGDC), this enzymatic complex is known for its role in the citric acid cycle. Three different forms of this complex can be identified based on the specific substrate of interaction. One form is specific for pyruvate, a second one is specific for 2-oxoglutarate, and a third one is specific for branched-chain  $\alpha$ -keto-acids. Because the same dehydrogenase subunit is utilized, the three forms of the complex utilized the same coenzymes: i.e. TTP, CoA, lipoate, FAD and NAD. Functionally, this dehydrogenase is involved in lysine degradation and tryptophan metabolism in addition to playing a key control point in the TCA cycle. In this context, the dehydrogenase controls the level of reducing equivalents, such as NADH, generated in the cycle which, in turn increase the electrons flux through the mitochondrial electron transport chain, enhancing oxidative phosphorylation and ultimately ATP synthesis [10]. In a classic enzymatic inhibitory feedback, the activity of  $\alpha$ -ketoglutarate dehydrogenase is inhibited by the reaction by-products succinyl-CoA and NADH, as well as by a high energy status (elevated ATP and NADH levels) within the cell, and by CoA-SH. Conversely, an increase in ADP level acts as an allosteric activator of the dehydrogenase. Calcium [10] and magnesium [11] ions also act as allosteric activators of the enzyme. The effects of  $Ca^{2+}$  and  $Mg^{2+}$  on the enzyme activity appear to be additive when the concentrations of free  $Mg^{2+}$  in the matrix is  $< 1$  mM. The presence of the divalent cations markedly decrease the  $K_m$  for  $\alpha$ -ketoglutarate from  $\sim 4$ – $5$  mM in the absence of  $Ca^{2+}$  and  $Mg^{2+}$ , to  $2.2$  mM in the presence of  $Ca^{2+}$  alone, to  $0.3$  mM in the presence of both cations [11]. The effect of  $Mg^{2+}$  is only observed in the presence of thiamine pyrophosphate (TPP), suggesting that the enzyme requires both TPP and  $Mg^{2+}$  for maximal activity. The presence of both cations also decreases the affinity of the dehydrogenase for  $NAD^+$  [11]. The modulatory effect of both  $Ca^{2+}$  and  $Mg^{2+}$  are observed at concentrations that are well within the range reported to occur in mitochondria for these cations under stimulatory conditions, e.g. by catecholamine or other hormones, thus supporting the likelihood that these regulatory effects can occur under in vivo conditions.

A side product of  $\alpha$ -ketoglutarate activity is the generation of free radicals, which can lead to oxidative stress if accumulating in high levels due to increased production and/or reduced detoxification. Due to its ability to generate free radicals, this dehydrogenase is considered de facto a mitochondrial redox sensor [12] in that it can modulate the rate of mitochondrial functioning and consequently the level of oxidative stress, thereby limiting the associated damage [12]. Under conditions in which the level of free radicals becomes extremely elevated, the enzyme can undergo a fully reversible oxidative inhibition [13]. This temporary inhibition

appears to occur through the reversible glutathionylation of the E2-lipoic acid domain of the dehydrogenase [14], thereby protecting the E2 domain, and the catalytic site of the dehydrogenase, from damaging oxidative stress. As the flux of electrons through the electron chain decreases, so does the production of free radicals, optimizing the conditions for the mitochondrial detox systems to scavenger these toxic agents.

*Isocitrate Dehydrogenase* (1.1.1.42 and 1.1.1.41): This enzyme catalyzes the oxidative decarboxylation of isocitrate to generate  $\alpha$ -ketoglutarate and  $\text{CO}_2$  in the citric acid cycle. The reaction is a two-step process, supported by the conversion of  $\text{NAD}^+$  to NADH. The mitochondrial isoform of this enzyme, IDH3, is a heterotetramer composed by two alpha, one beta, and one gamma subunits ( $2\alpha 1\beta 1\gamma$ ). Two other isoforms (IDH1 and IDH2) of the enzyme are known in humans. They catalyze the same reaction in the cytosol and in peroxisomes converting  $\text{NADP}^+$  to NADPH in the process.

Owing to the large negative free energy change involved in the reaction, the step catalyzed by this dehydrogenase represents one of the irreversible reactions in the citric acid cycle. Hence, tight regulation is required to avoid unnecessary depletion of isocitrate. The reaction is promoted by substrate availability and the presence of cofactors such as  $\text{NAD}^+$  and  $\text{Mg}^{2+}$  (or  $\text{Mn}^{2+}$ ), which both bind specific active sites on the IDH structure, and inhibited by ATP levels [15].

*Succinate dehydrogenase* (1.3.5.1): This enzyme participates in two key cycles within the mitochondrion. It is part of the respiratory complex II (whereby it is also defined as succinate-coenzyme Q reductase) and as such is key in controlling the electron flux through the electron transport chain [16]. In addition, it participates in the citric acid cycle, in which it catalyzes the oxidation of succinate to fumarate with the reduction of ubiquinone to ubiquinol.

Structurally, the succinate dehydrogenase is composed of 2 hydrophilic and 2 hydrophobic subunits. The two hydrophilic subunits possess a covalently attached flavin adenine dinucleotide (FAD) cofactor and the succinate binding site (subunit SdhA) and three iron-sulfur clusters (SdhB). The subunit SdhA provides the binding site for the oxidation of succinate. The two hydrophobic subunits SdhC and SdhD act as membrane anchors. Human mitochondria contain 2 distinct SdhA isoforms (FpI and FpII), which form a membrane bound cytochrome b complex with 1 heme b group and 1 ubiquinone binding site. More specifically, the ubiquinone binding site is located in an enclosure formed by SdhB, and the two hydrophobic subunits SdhC and SdhD. These two subunits also present one cardiolipin and one phosphatidylethanolamine molecule attached [17].

Functionally, the succinate-binding site in SdhA and the ubiquinone-binding site in the pocket formed by the three other subunits are connected by a chain of redox centers that include FAD and the three iron-sulfur clusters [17]. Due to its localization, the succinate dehydrogenase participates to both the citric acid cycle and the respiratory chain, as indicated previously. Electron removed from succinate during its oxidation to fumarate are transferred through the iron-sulfur clusters present in SdhB to the ubiquinone molecule, and ultimately to the two hydrophobic subunits anchoring the complex to the mitochondrial membrane. The acceptance of the electrons by the ubiquinone occurs in a two-step process whereby, after the first electron transfer, the ubiquinone is converted to a semiquinone radical species. The transfer of the second electron fully converts this intermediate to ubiquinol [18]. Hence, SdhA acts as an intermediate in the enzymatic activity of the succinate dehydrogenase complex in that:



1) it converts succinate to fumarate as part of the citric acid cycle while converting FAD to FADH<sub>2</sub>; 2) electrons from FADH<sub>2</sub> are transferred to the iron–sulfur clusters of the SdhB subunits as part of the respiratory chain function, and 3) the electrons are ultimately transferred to ubiquinone Q via the hydrophobic subunits of the complex. Inhibition of the succinate-ubiquinone activity results in the transfer of the electrons flowing through the SdhB subunit to O<sub>2</sub>, thereby generating reactive oxygen species (e.g. superoxide). Accumulation of reactive oxygen species stabilizes the production of HIF-1 $\alpha$ , and facilitating its interaction with HIF-1 $\beta$  to form a critical heterodimeric complex that induces the expression of anti-apoptotic genes but also tumor-causing genes (see section 3 for further details). Under conditions in which succinate dehydrogenase activity is inhibited, succinate accumulates within the mitochondria and then diffuses into the cytoplasm where it inhibits the physiological hydroxylation of HIF-1 $\alpha$  in the cytosol by prolyl-hydroxylase (PHD). Inhibition of HIF-1 $\alpha$  hydroxylation works in conjunction with the accumulation of reactive oxygen species occurring through the succinate dehydrogenase complex to stabilize HIF-1 $\alpha$  and promote the formation of a stable and active HIF complex that promotes the expression of tumor-inducing genes [19]. Because PHD activity requires oxygen,  $\alpha$ -ketoglutarate as substrates, and ferrous iron and ascorbate as co-factors, increasing  $\alpha$ -ketoglutarate levels could represent a viable therapeutic approach to limit tumor development and growth under SDH deficiency.

*Pyruvate dehydrogenase* (1.2.4.1): This enzyme is the first component of the pyruvate dehydrogenase complex (PDC), which is responsible for transforming pyruvate to acetyl-CoA via pyruvate decarboxylation. The Acetyl-CoA generated by the reaction then enters the citric acid cycle, contributing to cell respiration. As a result, pyruvate dehydrogenase links glycolysis to the citric acid cycle and the release of energy via NADH. The complex is constituted by the pyruvate dehydrogenase (E1) component, a dihydrolipoamide acetyltransferase (E2) component, a pyruvate dehydrogenase kinase (PDK) and a pyruvate dehydrogenase phosphatase. The reaction operated by E1 uses thiamine pyrophosphate (TPP) as a required cofactor, and it is considered to be the rate-limiting step for the whole pyruvate dehydrogenase complex (PDHc) activity. Phosphorylation of E1 by PDK inactivates E1 and consequently the whole complex. Pyruvate is a natural inhibitor of PDK, thereby resulting in a higher level of active, unphosphorylated PDH [20]. Alternatively, the phosphorylation state of E1 is reversed by the activity of pyruvate dehydrogenase phosphatase, which is stimulated by insulin, phospho-enol-pyruvate, and AMP, and competitively inhibited by ATP, NADH, and Acetyl-CoA. Physiological fluctuations in mitochondrial Ca<sup>2+</sup> and Mg<sup>2+</sup> levels, as observed following insulin stimulation, also stimulate the phosphatase activity, maintaining E1 in the active state [20].

### 3. Mitochondrial dehydrogenases and cancer

Cancer cells are characterized by increased glycolytic ATP production as a result of decreased mitochondria effectiveness. Inhibition (or decreased activity) of the citric acid cycle is considered to be one of the main causes forcing the cells to generate ATP through anaerobic glycolysis [21]. This reprogramming results from oncogene activation or inhibition of tumor suppressors [21]. Consistent with this observation, inhibition of glycolysis by dichloroacetate,

a pyruvate inhibitor, shifts cell metabolism back to oxidative phosphorylation, at least to a certain extent [22]. This metabolic reprogramming is considered to be necessary to meet the needs of the rapid proliferative rate exhibited by cancer cells. Two key bioenergetics parameters resulting from the mentioned metabolic reprogramming are anaerobic glycolysis, with associated extracellular acidification, and mitochondrial respiration.

Altered functioning of any of the mentioned dehydrogenases has been observed in different cancer cells, and specific correlations have been observed and documented. For example, glutamate dehydrogenase can be considered as biomarkers for cancer cell growth [23]. Similarly, defects in isocitrate dehydrogenase [24],  $\alpha$ -ketoglutarate dehydrogenase [25] succinate dehydrogenase [26], and pyruvate dehydrogenase [27] have all been reported in a variety of cancer cells, and associated with the metabolic reprogramming these cells undergo. The picture that emerges is that many cancer cells are hypoxic and therefore metabolize lactate to pyruvate to generate ATP [28]. The occurrence of hypoxia results in the activation of hypoxia-inducible factor 1 (HIF-1) heterodimeric DNA-binding complex and pro-neoplastic genes in tumor cells. HIF-1 $\alpha$  is continuously synthesized and degraded, with a half-life of ~6 min under normoxic conditions. Under hypoxia conditions, however, the rate of HIF-1 $\alpha$  degradation decreases significantly as a result of prolyl hydroxylation and proteosomal degradation suppression by accumulating succinate and increasing ROS formation and enzyme modification. As a result, HIF-1 heterodimer accumulates, and translocated to the nucleus of the cells where it activates genes responsible for increased glucose uptake (mostly GLUT1) and lactate production. At the same time, the increase in succinate levels further depresses mitochondrial respiration, with increased routing of O<sub>2</sub> towards ROS formation. In addition to upregulating the glucose transporter, HIF-1 promotes the expression of various glycolytic enzymes as well as PDK-1, the kinase that phosphorylates and inactivates the pyruvate dehydrogenase subunit E1. Inactivation of this dehydrogenase prevents pyruvate entry into the TCA cycle, thus down-regulating mitochondria respiration [29].

Another pathway involved in modulating mitochondria activity and responsiveness in cancer cells is the one tapping onto peroxisome proliferator-activated receptor gamma co-activator 1 $\alpha$  (PGC1 $\alpha$ ). This pathway has been observed to be overexpressed in some subsets of melanomas in which it activates mitochondrial oxidative phosphorylation and TCA-cycle specific genes for the metabolic needs of the cancer cells [30]. In addition, PGC1 $\alpha$  promotes the expression of genes involved in *de novo* lipogenesis and in the pentose phosphate pathway, increasing NADPH production for fatty acid synthesis purposes [30]. While genes involved in oxidative phosphorylation and TCA cycle are located in mitochondria, those involved in the pentose phosphate pathway and fatty acid synthesis are located in the cytoplasm of the cell. How exactly Acyl-CoA generated within the mitochondrion through aerobic glycolysis (pyruvate dehydrogenase) is utilized to enhance fatty acid synthesis is not completely understood. Experimental evidence would suggest that PGC1 $\alpha$  may do so by inducing the expression of genes responsible for converting citrate back to oxaloacetate and Acyl-CoA [30]. Irrespective of the precise mechanisms involved in the metabolic reprogramming of cancer cells, PGC1 $\alpha$ -positive cancer cells are particularly sensitive to pharmacological (e.g. metformin) or chemical (e.g. rotenone or FCCP) inhibitors of oxidative phosphorylation, in stark contrast to PGC1 $\alpha$ -negative cancer cells, which are more sensitive to anti-glycolytic agents (e.g. 2-deoxyglucose, or 2-DG).

*Mitochondrial ROS and Cancer:* Reactive oxygen species (ROS) are by-products of mitochondrial electron transport chain, generated by the incomplete reduction of oxygen as electrons flow from one complex to the next. Under physiological conditions, 1–2% of the molecular oxygen utilized by the mitochondria is converted to ROS [31]. Reactive oxygen species are highly reactive molecules, which act as oxidants removing electrons from DNA, proteins, and lipids. While ROS can be generated at the level of the plasma membrane (NADPH oxidase) and the endoplasmic reticulum of cell abundant in cytochrome activity (e.g. hepatocytes), the main intracellular source of ROS in most cells is indeed represented by mitochondria. In particular,  $O_2^-$  is the main byproduct of oxidative phosphorylation, and acts as precursor of other ROS products such as hydrogen peroxide ( $H_2O_2$ ) and hydroxyl radical ( $OH^\cdot$ ). Due to its negative charge,  $O_2^-$  cannot diffuse across biological membrane. However, evidence suggests that it can cross the mitochondrial membrane and diffuse into the cytoplasm through VDAC and other not-yet identified mitochondrial channels [32]. Either in the mitochondria or in the cytoplasm superoxide dismutases convert  $O_2^-$  to  $H_2O_2$ , which can now diffuse freely across membranes.  $H_2O_2$  is highly reactive towards cysteine residues, and this reactivity is further enhanced in the presence of  $O_2^-$  and iron or other transition metals (Fenton reaction or Haber-Weiss reaction), generating the more reactive but short-lived  $OH^\cdot$ , which interacts with any surrounding macromolecule, including proteins, lipids, nucleic acids, and carbohydrates [33]. Interaction of ROS with surrounding macromolecules may result in damage of biological membrane, enzyme inactivation, and genotoxicity. High levels of ROS can induce apoptosis and cell death whereas low levels of ROS can act as signaling molecules, and either path can results in tumor initiation and progression.

Cellular DNA and mitochondrial DNA are both highly susceptible to ROS-induced damage. This susceptibility is more pronounced for mitochondrial DNA, due to the close proximity to the electron transport chain where ROS are generated, the absence of protective histones, and the limited DNA repair capability. As a result, the mutation rate of mitochondrial DNA is two orders of magnitude higher than that of nuclear DNA. Consistent with this notion, mutations in mitochondrial DNA - in particular mutations of oxidative phosphorylation enzymes - have been observed in many neoplastic cells (see previous section), in which they result in oxidative phosphorylation dysfunction, increased ROS formation, and energetic shift towards anaerobic glycolysis.

The role of ROS as signaling molecules in cell proliferation, differentiation, migration, metastatic colonization, and gene transcription is now fairly well recognized. The ability of ROS to act as signaling molecules depends on the presence of redox-sensitive proteins that operate as 'ROS sensors'. In most cases, the sensing ability lies in the reversible oxidation of sulfhydryl groups in specific cysteine residues by  $H_2O_2$  or other ROS, resulting in the inhibition of that particular protein. For the most part, this inhibition is transient in that the cell possesses scavenging mechanisms (e.g. glutathione) to reduce the sulfhydryl groups back to the original state, thus restoring the protein's activity or signaling properties [34].

The levels of ROS produced by tumor cells are usually higher than those present in normal cells, and they results in DNA damage but also in increased tumorigenesis and metastasis via direct activation of signaling pathways [35]. Two signaling pathways activated by ROS are MAP Kinase and phosphoinositide 3-kinase, which both control cell survival and proliferation. The



upregulation of these pathways increases the expression of oncogenes and proteins involved in metastasis (e.g. matrix metalloproteinases) and in epithelial to mesenchymal transition [36]. Of note, oncogene activation has been reported to enhance mitochondrial ROS production, and ROS generation appears to be required for oncogene-mediated cell transformation [37].

#### 4. Mitochondrial dehydrogenases under diabetic conditions

A vast body of literature covers the functional modifications of mitochondria under diabetic conditions. Because cardiovascular insults remain the leading cause of death for diabetic patients [38], most of the attention has been focused on the modifications occurring in cardiac mitochondria to provide a better rationale for the morphological and functional modifications observed in diabetic hearts. Further, the incidence and severity of cardiovascular complications are markedly increased in both male and female diabetic patients, with the latter exhibiting a greater incidence than the diabetic male counterparts [39].

In humans, our understanding of the disease is complicated by the presence of two pathological conditions: diabetes type 1, in which insulin production is lacking, and diabetes type 2, in which insulin is still produced, sometimes to a greater extent than under normal conditions, and yet it is not functionally efficient in controlling the glycemic state of the patient. An additional confounding parameter is that to a large extent type 2 diabetic patients are overweight or obese, with an altered lipid status both in serum and within tissues (lipotoxicity).

This variety of conditions is also reflected in the heterogeneity of animal experimental models utilized to investigate the disease. Also here, models of type 1 diabetes are conceptually simpler in that animals are injected with agents that damage pancreatic beta-cells and abolish insulin secretion, fully mimicking the human condition. Models for type 2 diabetes are more heterogeneous, spanning from genetically conditioned animals (KO animals for specific proteins, receptors, or signaling molecules) to genetically inbred animals (e.g. Koletsky rats), to animals that develop diabetes spontaneously, to animals fed assorted high fat diets with or without sucrose, and injected with subliminal doses of beta-cell damaging agents that limit but not completely abolish the endogenous production of insulin, or treated with various oral antidiabetic agents [40]. Because of this plethora of models, it is not simple to determine to which extent modifications of mitochondrial dehydrogenase are a primary or secondary insult in the pathogenesis of the diabetic condition and its complications.

The development of diabetic cardiomyopathy has been etiologically attributed to several factors including metabolic [41], biochemical [42] and ultra-structural [43] modifications within the cardiac myocytes. Scrutiny of mitochondrial function in cardiac mitochondria from streptozotocin-treated rats has shown a decline in respiration and oxygen consumption, more pronounced for state 3 than state 4, which translates into a decrease in ATP production [44]. This energy deficiency has been considered a key factor in the development of diabetes-related cardiac dysfunctions, although changes in cardiac microvasculature [45], metabolic and hormonal disturbances [46] and concurring hyperglycemia [47] can certainly act as co-factors. Combining defective mitochondrial respiration with increased ROS formation, studies from our laboratory have indicated that succinate dehydrogenase [48] and pyruvate dehydrogenase [49] are highly sensitive to ROS-induced damage, forming stable, non-functional adducts

within the mitochondrion of streptozotocin-treated rats. As mentioned in the previous section, the consequences of this dysfunction are three-fold: 1) the inability of the electron transport chain to operate properly is compromised, resulting in decreased ATP production; 2) the production of ROS is enhanced, further damaging macromolecules within the mitochondrion and its surroundings, and 3) forcing the cardiac myocytes to depend on glycolysis to produce ATP. Interestingly, these changes were observed predominantly in cardiac and liver tissue but not in the kidney of the diabetic animals [49], rising the intriguing question of which protective mechanism(s) may operate in the latter organ under diabetic conditions.

## 5. Mitochondrial dysfunction in obesity

The term obesity refers to a condition in which the amount of fat tissue in the body is increased to an excessive degree (i.e. more than 25% in body weight in men and more than 30% in women). The incidence of obesity or just overweight has been on the rising for the last several decades, and currently affects anywhere between 35 and 45% of the population, in developed and developing countries [50]. Obesity is the sixth most important risk factor, and the number of affected individuals has reached 2.1 billion worldwide, including 10% of all children. The main complications include particular forms of cancer (e.g. colon and breast cancer, in particular), type 2 diabetes mellitus, cardiovascular pathologies including stroke, and musculoskeletal dysfunctions, with a prohibitive price tag in terms of healthcare costs, morbidity and mortality [51]. Several reviews have addressed the changes in mitochondria functioning in obesity, and we refer to them for an in-depth understanding of the changes occurring in the organelle [52–53]. The emerging picture indicates that cardiac cells, adipocytes, skeletal muscle cells, beta-cells, liver cells and others are affected to a varying degree by lipotoxicity [54], which also affects mitochondria operation. Whether the dysfunction strictly depends on the abnormal presence of fatty acid in the cells and the biological membrane of the organelle, or other not-yet identified factors remains undefined. The most common mitochondrial dysfunctions associated with obesity have been identified in an abnormally low number of mitochondria with altered morphology, decreased expression of the F1-ATPase subunit, with consequent low ATP generation [53], higher expression of the uncoupling protein 2 (UCP2) [52] with associated increased production of  $O_2^-$ , ROS in general, and apoptosis [52]. At the same time, the abnormal presence of fatty acids and ceramide within the cell activate specific signaling that further impair the tissue response to insulin [53]. Due to the decline in number and the altered morphology of mitochondria, an unanswered question remains as to whether these modifications are secondary to intrinsic defects in mitochondrial dehydrogenases that link electron transport chain to TCA cycle and/or to proper ATP synthesis, or whether defects in mitochondria enzymes are consequence of defective synthesis of key components within the cell nucleus and defective import into already altered organelles.

## 6. Conclusions

The picture we have attempted to draw in the present review moves from the important role specific mitochondrial dehydrogenases play in cell metabolism. Under conditions in which metabolic reprogramming occurs (i.e. cancer, diabetes, obesity), cells move away from efficient

mitochondrial bioenergetics and come to rely on glycolysis for ATP generation purposes. This metabolic shift has the unintended consequence of increasing reactive oxygen species production, which further disrupt cell metabolism and activate genes and oncogenes through specific signaling pathways and nuclear transcription factors (HIF-1 $\alpha$ , MAPKs, PI3K). Current and future lines of research aim at better understanding the signaling and metabolic routes connecting mitochondria to cell functions in the attempt to possibly recondition metabolism and energy production of target cell and mitochondria.

## Acknowledgements

This study was supported by NIAAA-11593, HL 090969, and departmental fundings to Dr. A. Romani.

## Author details

Andrea M.P. Romani

Address all correspondence to: amr5@po.cwru.edu

Department of Physiology and Biophysics, School of Medicine, Case Western Reserve University, Cleveland, OH, United States

## References

- [1] Henle J. Allgemeine Anatomie. Leipzig; 1841
- [2] Altmann R. Die Elementarorganismen und ihre Beziehungen zu den Zellen. Leipzig: Veit; 1890
- [3] Benda C. Ueber die Spermatogenese der Vertebraten und höherer Evertbraten. II. Theil: Die Histiogenese der Spermien. Archives of Analytical Physiology. 1898;393-398
- [4] Regaud C. Comptes Rendus Des Seances De La Societe De Biologie Et De Ses Filiales. 1908;65:718-720
- [5] Palade GE. Proceedings of National Academy of Sciences U.S.A. 1964;52:613-634
- [6] Ernster L, Schatz G. Mitochondria: A historical review. The Journal of Cell Biology. 1981;91:227-255
- [7] McBride HM, Neuspiel M, Wasiak S. Mitochondria: More than just a powerhouse. Current Biology. 2006;16:R551-R560
- [8] Mitchell P. Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation. Bodmin: Glynn Research . Glynn Research Ltd; 1966. p. 192

- [9] McKenna MC, Ferreira GC. The glutamate/ GABA-glutamine cycle. In: Enzyme Complexes Important for the Glutamate-Glutamine Cycle. Springer Int; 2016. pp. 59-98
- [10] Tretter L, Adam-Vizi V. Alpha-ketoglutarate dehydrogenase: A target and generator of oxidative stress. *Philosophical Transactions of the Royal Society B: Biological Sciences*. 2005;**360**:2335-2345
- [11] Panov A, Scarpa A. Independent modulation of the activity of alpha-ketoglutarate dehydrogenase complex by  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ . *Biochemistry*. 1996;**16**:427-432
- [12] McLain AL, Szweda PA, Szweda LI.  $\alpha$ -Ketoglutarate dehydrogenase: A mitochondrial redox sensor. *Free Radical Research*. 2011;**45**:29-36
- [13] McLain AL, Cormier PJ, Kinter M, Szweda LI. Glutathionylation of  $\alpha$ -ketoglutarate dehydrogenase: The chemical nature and relative susceptibility of the cofactor lipoic acid to modification. *Free Radical Biology & Medicine*. 2013;**61**:161-169
- [14] Applegate MA, Humphries KM, Szweda LI. Reversible inhibition of alpha-ketoglutarate dehydrogenase by hydrogen peroxide: Glutathionylation and protection of lipoic acid. *Biochemistry*. 2008;**47**:473-478
- [15] Tadhg PB, McMurry J. *The Organic Chemistry of Biological Pathways*. Roberts and Co. Publishers; 2005. pp. 189-190
- [16] Oyedotun KS, Lemire BD. The quaternary structure of the *Saccharomyces cerevisiae* succinate dehydrogenase. Homology modeling, cofactor docking, and molecular dynamics simulation studies. *The Journal of Biological Chemistry*. 2004;**279**:9424-9431
- [17] Yankovskaya V, Horsefield R, Törnroth S, Luna-Chavez C, Miyoshi H, Léger C, Byrne B, Cecchini G, Iwata S. Architecture of succinate dehydrogenase and reactive oxygen species generation. *Science*. 2003;**229**:700-704
- [18] Horsefield R, Yankovskaya V, Sexton G, Whittingham W, Shiomi K, Omura S, Byrne B, Cecchini G, Iwata S. Structural and computational analysis of the quinone-binding site of complex II (succinate-ubiquinone oxidoreductase): A mechanism of electron transfer and proton conduction during ubiquinone reduction. *The Journal of Biological Chemistry*. 2006;**281**:7309-7316
- [19] Selak MA, Armour SM, MacKenzie ED, Boulahbel H, Watson DG, Mansfield KD, Pan Y, Simon MC, Thompson CB, Gottlieb E. Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF- $\alpha$  prolyl hydroxylase. *Cancer Cell*. 2005;**7**:77-85
- [20] Jaimes R. Functional response of the isolated, perfused normoxic heart to pyruvate dehydrogenase activation by dichloroacetate and pyruvate. *Pflügers Archiv*. 2015;**468**:131-142
- [21] Ward PS, Thompson CB. Metabolic reprogramming: A cancer hallmark even Warburg did not anticipate. *Cancer Cell*. 2012;**21**:297-308
- [22] Michelakis ED, Sutendra G, Dromparis P, Weber L, Haromy A, Niven E, Maguire C, Gammer TL, Mackey JR, Fulton D, Abdulkarim B, McMurtry MS, Petruk KC. Metabolic modulation of glioblastoma with dichloroacetate. *Science Translational Medicine*. 2010; **2**:31ra34



- [23] Friday E, Oliver R, Welbourn T, Turturro F. Role of glutamate dehydrogenase in cancer growth and homeostasis. In: Canuto RA, editor. *Biochemistry, Genetics and Molecular Biology: Dehydrogenases*. 2012. DOI: 10.5772/48606
- [24] Reitman ZJ, Yan H. Isocitrate dehydrogenase 1 and 2 mutations in cancer: Alterations at a crossroads of cellular metabolism. *Journal of the National Cancer Institute*. 2010;**102**: 932-941
- [25] Vatrinet R, Leone G, De Luise M, Girolimetti G, Vidone M, Gasparre G, Porcelli AM. The  $\alpha$ -ketoglutarate dehydrogenase complex in cancer metabolic plasticity. *Cancer & Metabolism*. 2017;**5**:3
- [26] Ricketts CJ, Brian Shuch B, Vocke CD, Metwalli AR, Bratslavsky G, Middleton L, Yang Y, Wei M-H, Pautler SE, Peterson J, Stolle CA, Berton Zbar B, Merino MJ, Schmidt LS, Pinto PA, Srinivasan R, Karel Pacak K, Linehan WM. Succinate dehydrogenase kidney cancer: An aggressive example of the Warburg effect in cancer. *J. Urology*. 2012;**188**:2063-2071
- [27] Bingham PM, Zachar Z. The pyruvate dehydrogenase complex in cancer: implications for the transformed state and cancer chemotherapy. In: Canuto RA, editor. *Biochemistry, Genetics and Molecular Biology: Dehydrogenases*. 2012. DOI: 10.5772/48582
- [28] Valder-Helden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: The metabolic requirements of cell proliferation. *Science*. 2009;**324**:1029-1033
- [29] Weideman A, Johnson BS. Biology of HIF-1 $\alpha$ . *Cell Death and Differentiation*. 2008; **15**:621-627
- [30] Bhalla K, Hwang BJ, Dewl RE, Ou L, Twaddel W, Fang HB, Vafal SB, Vasquez F, Puigserver P, Boros L, Giron GD. PGC1 $\alpha$  promotes tumor growth by inducing gene expression programs supporting lipogenesis. *Cancer Research*. 2011;**71**:6888-6898
- [31] Ott M, Gogvadze V, Orrenius S. Mitochondria, oxidative stress and cell death. *Apoptosis*. 2007;**12**:913-922
- [32] Han D, Antunes F, Canali R. Voltage dependent channels control the release of the superoxide anion from mitochondria to cytosol. *The Journal of Biological Chemistry*. 2003;**278**:5557-5563
- [33] D'Autreaux B, Toledano MB. ROS as signaling molecules: Mechanisms that generate specificity in ROS homeostasis. *Nature Reviews. Molecular Cell Biology*. 2007;**8**:813-824
- [34] Meng TC, Fukada T, Tonks NK. Reversible oxidation and inactivation of protein tyrosine phosphatase in vivo. *Molecular Cell*. 2002;**9**:387-399
- [35] Weinberg F, Chandel NS. Reactive oxygen species-dependent signaling regulates cancer. *Cellular and Molecular Life Sciences*. 2009;**66**:3663-3673
- [36] Cannito S, Novo E, di Bonzo LV. Epithelial-mesenchymal transition. From molecular mechanisms, redox regulation to implications un human health and disease, *Antioxidants & Redox Signaling*. 2010;**12**: 1383-1430

- [37] Weinberg F, Hamanaka R, Wheaton WW. Mitochondrial metabolism and ROS generation are essential for Kras-mediated tumorigenicity. *Proceedings of National Academy of Sciences USA*. 2010;**107**:8788-8793
- [38] World Health Organization. Global Status Report on Non-Communicable Diseases 2010. Geneva: Switzerland; 2011
- [39] Kannel WB, McGee DL. Diabetes and cardiovascular risk factors: A Framingham study. *Circulation*. 1979;**59**:8-13
- [40] Cefalu WT. Animal models of type 2 diabetes: Clinical presentation and Pathophysiological relevance to the human condition. *ILAR Journal*. 2006;**47**:186-198
- [41] Rodrigues B, Cam MC, McNeill JH. Metabolic disturbances in diabetic cardiomyopathy. *Molecular and Cellular Biochemistry*. 1998;**180**:53-57
- [42] Pierce GN, Kutryk MJ, Dhalla NS. Alterations in Ca<sup>2+</sup> binding by and composition of the cardiac sarcolemma membrane in chronic diabetes. *Proceedings of National Academy of Sciences USA*. 1983;**80**:5412-5416
- [43] Dillmann WH. Diabetes mellitus induces changes in cardiac myosin of the rat. *Diabetes*. 1980;**29**:579-582
- [44] Pierce GN, Dhalla NS. Heart mitochondrial function in chronic experimental diabetes in rats. *The Canadian Journal of Cardiology*. 1985;**1**:48-54
- [45] Factor SM, Okun EM, Minase T. Capillary microaneurysms in the human diabetic heart. *The New England Journal of Medicine*. 1980;**302**:384-388
- [46] Fein FS, Kornstein LB, Strobeck JE, Capasso JM, Sonnenblick EH. Altered myocardial mechanics in diabetic rats. *Circulation Research*. 1980;**47**:922-933
- [47] Rubler S, Dlugash J, Yuceoglu YZ, Kumral T, Branwood AW, Grishman A. New type of cardiomyopathy associated with diabetic glomerulosclerosis. *The American Journal of Cardiology*. 1972;**30**:595-602
- [48] Lashin O, Szweda PA, Szweda LI, Romani AMP. Decreased complex II respiration and HNE-modified SDH subunit in diabetic heart. *Free Radicals Biology & Medicine*. 2005;**40**(2006):886-896
- [49] DiNuscio G, Yuan C, Lashin O, Romani A. Identification of specific mitochondrial proteins forming stable adducts with 4-hydroxynonenal within cardiac tissue of type-i diabetic animals: implications for bioenergetics dysfunction and onset of diabetic cardiomyopathy. In: Osterhoudt G, Barhydt J, editors. *Cell Respiration and Cell Survival*. Nova Science Publishers, Inc; 2009
- [50] <http://www.cdc.gov/obesity/data/adult.html>
- [51] Haslam DW, James WP. Obesity. *Lancet*. 2005;**366**:1197-1209

- [52] Wang B-D, Rennert OW, Su YA. Mitochondrial oxidative Phosphorylation. Obesity and Diabetes, Cellscience Reviews 4. 2008
- [53] Sivitz WI. Mitochondrial dysfunction in obesity and diabetes. US Endocrinology. 2010;**6**:20-27
- [54] Medina-Gomez G, Gray S, Vidal-Puig A. Adipogenesis and lipotoxicity: Role of peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) and PPAR $\gamma$ -coactivator-1 (PGC-1). Public Health Nutrition. 2007;**10**:1132-1137